PA NT COOPERATION TREAT

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 26 February 2001 (26.02.01)	
International application No. PCT/SE00/01174	**Applicant's or agent's file reference** P4755PC/Ali
International filing date (day/month/year) 21 June 2000 (21.06.00)	**Priority date** (day/month/year) 30 June 1999 (30.06.99)
Applicant NORRMAN, Nils	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   22 January 2001 (22.01.01)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

R. E. Stoffel

Telephone No.: (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01174

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/543, C12Q 1/68
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N, C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EO 0773227 A1 (AFFYMAX TACHNOLOGIES N.V.), 16 Sept 1992 (16.09.92), page 5, line 17 - line 33, see examples 4 and 5 --	1-16
Y	US 5858670 A (KIT SANG LAM ET AL), 12 January 1999 (12.01.99), column 20 - column 23, figure 1, see the claims --	1-16
A	Biopolymers (Peptide Science), Volume 37, 1995, (Tucson), Michal Lebl et al, "One-Bead-One-Structure Combinatorial Libraries" page 177 - page 198 --	1-16

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 2000	06 -11- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl-Olof Gustafsson Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01174

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 9735198 A1 (ONTOGEN CORPORATION), 25 Sept 1997 (25.09.97), page 16; page 10	1-14
	--	
Y	WO 9528640 A1 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK), 26 October 1995 (26.10.95), page 61 - page 68	1-16
	--	
A	Reactive Polymers, Volume 22, 1994, Vered Hornik et al, "Self-encoded, highly condensed solid phase-supported peptide library for identification of ligand-specific peptides"	1-16
	--	
Y	WO 9622531 A1 (HÄNNINEN, PEKKA), 25 July 1996 (25.07.96), page 7 - page 8	1-16
	--	
Y	EP 0440193 A2 (FUJIREBIO INC.), 7 August 1991 (07.08.91)	1-16
	--	
A	Bioorganic & Medicinal Chemistry, Volume 5, No 3, 1997, (Great Britain), Hubert Maehr, "Combinatorial Chemistry in Drug Research from a New Vantage Point" page 473 - page 491	1-14
	--	
Y	National Library of Medicine (NML), file Medline, Medline accessio no. 95230083, Lam KS et al: "Application of a dual color detection scheme in the screening of a random combinatorial peptide library"; & J Immunol Methods 1995 Mar 27;180 (2):219-23	1-16
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/SE 00/01174

EO	0773227	A1	16/09/92	NONE			
US	5858670	A	12/01/99	AT	176330	T	15/02/99
				AU	659091	B	11/05/95
				AU	2836995	A	07/12/95
				AU	8238591	A	23/01/92
				CA	2086672	A	03/01/92
				CZ	9204073	A	13/10/93
				DE	69130828	D,T	17/06/99
				EP	0542770	A,B	26/05/93
				SE	0542770	T3	
				ES	2126572	T	01/04/99
				FI	925986	A	31/12/92
				GR	3029443	T	28/05/99
				HU	63576	A	28/09/93
				HU	9204179	D	00/00/00
				IL	98682	A	18/02/97
				JP	6500308	T	13/01/94
				KR	222146	B	01/10/99
				MX	9100052	A	28/02/92
				NO	930011	A	22/02/93
				NZ	238805	A	26/07/94
				PL	168354	B	29/02/96
				PL	169616	B	30/08/96
				RO	112336	A	29/08/97
				SK	407392	A	10/08/94
				US	5382513	A	17/01/95
				US	5510240	A	23/04/96
				US	5650489	A	22/07/97
				WO	9200091	A	09/01/92
				AU	683762	B	20/11/97
				AU	2845495	A	16/05/96
				ZA	9105113	A	27/05/92
WO	9735198	A1	25/09/97	AU	2537397	A	10/10/97
				CA	2249419	A	25/09/97
				EP	0904540	A	31/03/99
WO	9528640	A1	26/10/95	AU	2292695	A	10/11/95
				CA	2187792	A	26/10/95
				CN	1151793	A	11/06/97
				EP	0755514	A	29/01/97
				HU	74985	A	28/03/97
				HU	9602800	D	00/00/00
				JP	10502614	T	10/03/98
				NO	964332	A	03/12/96
				US	5565324	A	15/10/96
				US	5789172	A	04/08/98
				US	5968736	A	19/10/99
				US	6001579	A	14/12/99

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/SE 00/01174

WO	9622531	A1	25/07/96	EP	0804732	A	05/11/97
				FI	96641	B,C	15/04/96
				FI	950175	D,V	06/04/95
				JP	10512670	T	02/12/98
				US	5891738	A	06/04/99
				EP	0815447	A	07/01/98
				FI	101829	B	00/00/00
				FI	951040	A	08/09/96
				JP	11503824	T	30/03/99
				WO	9627798	A	12/09/96
EP	0440193	A2	07/08/91	AU	642459	B	21/10/93
				AU	7012091	A	01/08/91
				CA	2035305	A	01/08/91
				JP	3225277	A	04/10/91

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

3

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P4755PC/Ali		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/SE00/01174	21.06.2000	30.06.1999
International Patent Classification (IPC) or national classification and IPC7		
G 01 N 33/543, C 12 Q 1/68		
Applicant		
Amersham Pharmacia Biotech AB et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22.01.2001	28.08.2001
Name and mailing address of the IPEA/SE	Authorized officer
Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. 08-667 72 88	Telex 17978 PATOREG-S Carl-Olof Gustafsson/EÖ Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE00/01174

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☐ the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the claims:
pages _____, as originally filed
pages _____, as amended (together with any statement) under article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the drawings:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ **the language of publication of the international application (under Rule 48.3(b)).**

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE00/01174

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	10	YES
	Claims	1-9, 11-16	NO
Inventive step (IS)	Claims		YES
	Claims	1-16	NO
Industrial applicability (IA)	Claims	1-16	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The International Search Report revealed several documents considered to be of particular relevance:

1. EP773227 see page 5, lines 17-33, figs 1 and 2 and examples 4 and 5
2. US5858670, see cols. 20-23, fig. 1 and claims
3. WO9735198 see pages 10 and 16
4. WO9528640 see pages 51-57 and 61-80 and claims
5. WO9622531, see pages 7-8
6. EP440193
7. J. Immunol. Methods, Vol.180, 1995, pp 219-23, Medline abstr. 95230083

D1 pertains to screening of libraries of ligands (e.g. in the form of an oligomer) having identifier tags linked to particles. The tagged particle identifies the ligand ("oligomer") by e.g. a physical feature of the tag (size, shape, colour, p 5, l 17-20, "light addressable compounds" p 4, l 6). A preferred form of tag is a nucleotide sequence. The series of steps forming the individual ligand oligomers are recorded by tagging, each tag representing a step in the oligomer synthesis.
Thus, tagged subclasses of particles can be produced in a way that makes each subclass retrievable/identifiable by different physical properties. From the second step and on, subclasses may be produced that display two or more tags being distinguishable by their physical properties. At least the library according to claim 11-15 is therefore considered to lack novelty.

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

D4 reveals the use of physically distinguishable tags (p 51-3) in combinatorial chemical libraries. Obviously, a number of tags are associated with particular beads (p 57, l 30-31) for precise indication of the events on each class of beads or each bead. Analysis of tags can be achieved by physical methods such as chromatographic separations, spectrometry etc. Classes and subclasses of particles will evolve as soon as a number of particles with ligands have been produced and tagged. Assays may be performed on single particles by FACS or on groups of particles segregated into separate vessels and screened for released tags (see p 61-62, l 9). Thus D4 involves the distribution of particles into separate vessels in order to simplify the retrieval of particles bound to receptor/ligand and the identification of "class" and "subclass" of the identified and retrieved particle. The method according to claims 1-9 and the library according to claims 11-15 are therefore considered to lack novelty.

D5 maps multiple tagging biospecific assay methods used in identifying ligand binding compounds (see p 2-5), mentioning e.g. separate particle sizes, different fluorescent dyes etc. for tagging of classes of particles linked to separate ligands/receptors. The classes/categories of particles produced (and linked to different ligands/receptors) can be identified by "physical property" measurements.
D6 also pertains to

To the extent the method according to claim 1 is considered to be novel, it is nevertheless considered to be obvious to a person skilled in the art to apply the well known methods for multiple assays known from D5 in the library assays revealed in documents D1 and D4. Consequently, the method according to claims 1-10 and the library according to claims 11-16 are considered to lack an inventive step.

D2 relates to libraries of ligands on beads brought into contact with samples/cells in individual wells (chambers). No particular tags to be distinguished physically seem to be mentioned. D3 refers to spatially addressable libraries of ligands (e.g. oligomers). Although, tagging is eliminated with the spatial identification, These documents represent the state of the art.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 January 2001 (04.01.2001)

PCT

(10) International Publication Number
WO 01/01141 A1

(51) International Patent Classification[7]: G01N 33/543, C12Q 1/68

(74) Agent: DR LUDWIG BRANN PATENTBYRÅ AB; Box 1344, S-751 43 Uppsala (SE).

(21) International Application Number: PCT/SE00/01174

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9902479-6 30 June 1999 (30.06.1999) SE

(71) Applicant *(for all designated States except US)*: AMERSHAM PHARMACIA BIOTECH AB [SE/SE]; Björkgatan 30, S-751 84 Uppsala (SE).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: NORRMAN, Nils [SE/SE]; Sunnerstavägen 26 j, S-756 51 Uppsala (SE).

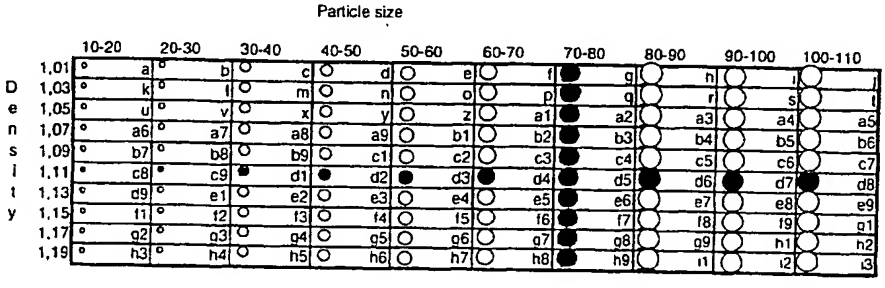
(81) Designated States *(national)*: JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: COMBINATORIAL LIBRARY WITH PARTICLE CLASSES THAT CAN BE DISTINGUISHED BY TWO FEATURES E.G. SIZE, DENSITY, COLOR, ETC.



(57) Abstract: The invention relates to a method of identifying one or more substances having affinity for a given target, comprising providing a set of particle classes, each said particle class being distinguishable from another class by at least one property. The particles belonging to one and the same class may have another property that distinguishes them, e.g. different densities, thus forming sub-classes. To each unique sub-class a number of unique ligands can be attached to sets of particles forming further sub-classes. A plurality of sub-classes is combined to form at least one mixture. The mixtures are distributed in separate vessels and exposed to said substance. All target substances not having bound to any candidate substance are washed away, and it is determined to which particle sub-class(es) said target substance actually has (have) bound.

WO 01/01141 A1

COMBINATORIAL LIBRARY WITH PARTICLE CLASSES THAT CAN BE DISTINGUISHED BY TWO FEATURES E.G. SIZE, DENSITY, COLOR, ETC.

The present invention relates generally to screening methods and selective identification of target substances.

Background of the invention

In the pharmaceutical area i.a. it is often necessary to test very large numbers of substances against one or more target compounds to find out if any of the tested substances possess affinity towards the target, or in some other way interacts with the target. This normally is performed by binding each candidate compound (as a ligand) to some matrix e.g. a particle, loading a number of the particles in one reaction vessel of some kind, and incubating with the target substance. Either the candidate compound (ligand) or the target is marked with identifiable groups such as fluorescent or radioactive groups or nuclei. After the reaction is deemed to have come to completion analysis of the vessel is performed to see if the marker substance or group is present, indicating that the target has indeed reacted with the substance bound to the matrix.

Screening large numbers of substances of course requires large numbers of vessels, which is laborious and tedious to handle. 10000 compounds would require 10000 individual reaction vessels to be handled.

Another possibility is to mark particles with say 10000 different ligands. A mixture is prepared comprising particles having 100 different ligands and the mixture is placed in a well of a microtiter plate. 100 such mixtures are prepared totaling 10000 different ligands and said mixtures are placed in one well each. All wells are exposed to and incubated with the target substance marked with a fluorescent moiety. If fluorescence is detected in one well one knows that a hit is present in that well. A second experiment is performed where the 100 different ligands in said well are individually placed in one well each, and the incubation and exposure is repeated. The well exhibiting fluorescence will then contain the desired substance. Thus, it is necessary to perform two sets of experiments.

Therefore, it would be desirable to have access to methods and means for rendering such screening procedures less time and space consuming, and to enable the procedure to be carried out in one step.

US-5,858,670 relates to identification by sequencing of peptides bound to beads. The method requires many operations. It mentions staining of beads (col. 41, lines 47-54) for identification. This particular aspect per se, does not form a part of the present invention

In "One-Bead-One-Structure Combinatorial Libraries", Biopolymers, Vol. 37, 177-198, there is disclosed a very complicated (many steps required) method of making libraries for the identification of binding to specific ligands. The actual screening procedure requires a large number of iterations.

US-5,817,751 discloses libraries where beads have "identifier tags". These tags can be "microscopically or otherwise distinguishable features", (size, shape, mass, charge, color). See col. 23, lines 10-21, and lines 42-46. The screening process involves releasing the tags from the beads and subsequent amplification thereof.

EP 0 773 227 A1 discloses "identifier tags", meaning some "physical attribute" (examples given are size, shape, color, optical density) by which a solid support (e.g. a bead) can be identified and distinguished. See p 5, lines 17-33. The screening procedure is conventional, and involves sorting out fluorescing beads by using a cell sorting instrument (FACS).

US-5,162,863 discloses the possibility of using particles of different sizes for identification purposes.

Summary of the Invention

The present invention seeks to provide a method of identifying one or more substances having affinity for a given target substance, that would require a smaller number of reaction vessels than what is necessary today, and also a smaller number of operations, thereby speeding up the process of investigation substantially.

This object is achieved by the method according to the invention, whereby a combination of distinguishable properties of particles to which ligands are attached, is used for identification of those particles to which a target substance has bound. The method is defined in claim 1.

By providing a class of particles having one property in common, and subdividing each such class into sub-classes having another property, it becomes possible to screen a very large number in only two steps.

In a second aspect of the invention there is provided a library of ligands, comprising individually distinguishable particles of a matrix material, having ligands attached to the surface, and being suitable for binding candidate compounds (ligands), and usable in screening procedures. This aspect is defined in claim 11.

A third aspect of the invention is the use in screening procedures, of a ligand library comprising individually distinguishable particles of a matrix material, suitable for binding candidate compounds, which is characterized by using fact that the particles are distinguishable, as a marker for each said candidate compound. The library is defined in claim 16.

Furthermore, in preferred embodiments, selecting the distinguishable property to be different particle size in combination with density, is very powerful in this respect.

Brief Description of the Drawings

The invention will now be described with reference to examples and to the drawings, in which

Fig. 1 is a schematic representation of a micro titer well in which a mixture of 100 different particle-ligand combinations has been incubated;

Fig. 2 is another schematic representation of a micro titer well;

Fig. 3 is photograph of the contents of a microtiter well taken through a fluorescence microscope; and

Fig. 4 is a graphical output from an image analysis.

Detailed Description of Preferred Embodiments

For the purpose of this application, the term "particle class" is taken to mean particles having at least one property distinguishing them from other particles of another "class". One such property can be size (diameter), another the density. Still another kind of "particle class" is formed when a number of particles of one class having a first ligand attached, is used together with particles of the same class but having a second ligand attached thereto, but where the ratio of the number of particles having different ligands is different, e.g. 2:1.

A further particle class is formed when a coordinate is assigned to a set of particles

The term "sub-class" is taken to mean particles having as common features, the features or properties of a "particle class", and having been treated in some way so as to be distinguishable from another "sub-class". A "sub-class" is e.g. formed when particles of one size are provided with one ligand, and another "sub-class" is formed when the same particles are provided with a second ligand, different from the first.

Within one and the same class, i.e. particles having the same size, "sub-classes" are also formed by providing particles with e.g. different density but having the same diameter.

The term "ligand" shall mean one moiety attached to a particle, or a group of such moieties.

The basic idea behind the invention is the insight that in a mixture of objects, wherein groups of objects in said mixture have some distinguishable and identifiable feature in common within each group, it is easy to identify each group of objects. Also it is easy to identify if there has been a change in the property of the objects in one group. If for example a mixture consists of all white golf balls, tennis balls and footballs, it is very easy by visual inspection to determine that say the golf balls have changed color to green.

This insight has been applied by the inventor in the field of screening for substances having some desired property amongst large numbers of candidate substances, or ligands.

An extremely simplified example is the case where two ligands are to be examined for affinity to a target substance suitably marked, e.g. with a fluorescent marker. Each ligand would then be bound to particles with mutually different size and then the particles are mixed. The mixture is placed in well of a micro titer plate and incubated. After incubation the well is washed. If the target selectively binds to one ligand it will be an easy matter to identify which ligand has bound since one can measure the size of the fluorescing particles and thereby determine which the ligand is.

As another simplified and illustrative but slightly more complex example, let us assume that one is interested in testing four ligands for affinity to some macro-molecule, e.g. a protein. Let us also assume that each different ligand is individually bound to a particle. Furthermore we assume that the particles are of only two different classes, in this case we assume two different particle sizes. Two mixtures are prepared, each comprising two sets of particles having distinguishable size, and having different ligands bound thereto. The two mixtures are placed in one well each and incubated with the macro-molecule, appropriately marked with e.g. a fluorescent marker. Thus, we have two wells each containing the same two different particle classes, but having mutually different ligands bound to them. After incubation the wells are washed to remove non-bound substances. If the macro-molecule binds to one ligand it will be a straight forward matter to identify which one by determining which particle size and which well exhibits fluorescence. This is most easily done under a fluorescence microscope, and can be done by ocular inspection in a case like the described, where there are only few wells and few ligands present. Other methods of detection are available, e.g. so called flow cytometry in combination with Coulter-counter techniques. These methods are well known to the skilled man and need not be discussed in detail herein.

For production screening using large numbers of ligands and particles in large numbers of wells, image analysis by computer using specialized software is more feasible. An example of such commercial software is LEICA® Q 500 MC.

The skilled man will appreciate that the principle demonstrated above is applicable to larger numbers of different particle sizes and large numbers of wells, as will be explained by further examples below.

The basic principle of a first embodiment of the invention may be said to encompass identification of a ligand having reacted with a target substance, by two parameters, namely a) particle size, and b) coordinate for the particle on the micro titer plate (simply in which well it is present).

Still another property of the particles that is usable as an identifying "marker" is the density. It is relatively easy to make particles having well defined densities. If ligands are bound to such particles, mixtures of said particles can be separated by centrifugation in a density gradient, and fluorescent bands in the test tube after centrifugation can be easily determined, and the position along the tube will indicate which density fraction is a hit.

The basic principle can be further elaborated by making mixtures containing different number ratios between particles of the same size having different ligands, and providing them in the same well. Thus, if two different ligands are bound to one and the same particle size but in two fractions, and these two fractions are mixed e.g. in a ratio 1:2, of course it will be an easy matter to determine to which fraction the target has bound by the fluorescence intensity being either 1/3 or 2/3 of the maximum possible, if all particles would have fluoresced.

The particles to be used may be made of any material to which suitable ligands of interest can be attached or bound. The particles are preferably hydrophilic and built up of one or more polymers which are insoluble in water. Hydrophobic polymers that have been derivatized to become hydrophilic are included. Suitable polymers are polyhydroxy polymers, e.g. based on polysaccharides, such as agarose, dextran, cellulose, starch, pullulan, etc. and completely synthetic polymers, such as polyacrylic amide, polymethacrylic amide, poly(hydroxyalkylvinyl ethers), poly(hydroxyalkylacrylates) and polymethacrylates (e.g. polyglycidylmethacrylate), polyvinylalcohols and polymers based on styrenes and divinylbenzenes, and copolymers in which two or more of the monomers corresponding to the above-mentioned polymers are included. Polymers, which are soluble in water, may be derivatized to become insoluble, e.g. by cross-linking and by coupling to an insoluble body

via adsorption or covalent binding. Hydrophilic groups can be introduced on hydrophobic polymers (e.g. on copolymers of monovinyl and divinylbenzene) by polymerization of monomers exhibiting groups which can be converted to OH, or by hydrophilization of the final polymer, e.g. by adsorption of suitable compounds, such as hydrophilic polymers.

The particles can also be based on inorganic material, such as silica. Preferred particles lack hydrolytically unstable groups, such as silane, ester and amide groups.

The particles may be porous.

The term "hydrophilic particle" in practice means that the accessible surface of the particles is hydrophilic in the sense that can be penetrated by aqueous liquids. Typically the accessible surfaces on a hydrophilic particle expose a plurality of polar groups for instance comprising oxygen and/or nitrogen atoms. Examples of such polar groups are hydroxy, amino, carboxy, ester, ether of lower alkyls (such as $(-CH_2CH_2O-)_nH$ where n is an integer).

Suitable size fractions of such particles are obtained by sieving, using standard methods well known to the skilled man. The resulting particle fractions will have some spread in terms of average diameter, but the spread can be controlled so that overlap between fractions can be adequately controlled.

Furthermore, a certain overlap in fraction size is no problem since the image analysis software that calculates average particle sizes is able to distinguish with a high degree of certainty to which fraction the fluorescing particles belong.

Another possible material for the particles is poly-styrene. By using this material it is possible to make mono-disperse particles without overlap in fraction size.

In order to prepare suitable mixtures when the number of particles and ligands is large, so called factor design is preferably used. The theory behind this methodology is disclosed in *"Statistics for Experiments"* by Box et al, ISBN 0-471-09315-7.

The invention will now be further described by way of the following non-limiting examples.

EXAMPLES

Example 1

10 particle fractions ranging in size from fraction 1 of 10-20 μm up to fraction 10 of 100-110 μm are prepared. Each size fraction is further subdivided into 10 density fractions ranging from 1,01 up to 1,19 with increments of 0,02. Thus, in all 100 different sub classes of particles defined by both a) size and b) density is produced. To particles of each sub class a different ligand is bound to provide 100 unique combinations of ligand and particle.

10000 different ligands are to be tested. Thus, the 100 particle subclasses are used to provide 100 sets of 100 particle-ligand combinations. Each set of 100 combinations is placed in one well each of a 100-well microtiter plate. Then all wells are incubated with a target compound. The compound is suitably marked with a fluorescent moiety. In addition to being identifiable by the two properties of the particle (size and density), each ligand is identified by a coordinate, i.e. the well number.

After incubation the wells are washed to remove all target compounds that have not bound to any ligands.

Fig. 1 shows a matrix symbolizing one well in which it has been determined that the size fraction 70-80 μm exhibits fluorescence (symbolized by the particles being filled), and thus contains a subclass of particles to which the target compound has bound. Of course only 10% of the particles of this size will fluoresce, but it cannot be decide which ligand has reacted.

In order to determine which density fraction contains the target bound to the particle, the entire contents in the well is centrifuged in a density gradient, so as to yield bands corresponding to each density. The band (density 1,11) containing the fraction having target bound thereto will then fluoresce. This is symbolized by the dots representing the density fraction in question being filled.

The cross section of the two rows will identify the particular ligand that has bound to the target molecule.

Example 2

In order to double the number of possible ligands to test (or alternatively to reduce the number of wells needed for the screening), it is also possible to use the number ratio between subclasses of particles as a marker.

Thus, 10 particle fractions ranging in size from fraction 1 of 10-20 μm up to fraction 10 of 100-110 μm are prepared. Each size fraction is further subdivided into 10 density fractions ranging from 1,01 up to 1,19 with increments of 0,02. Thus, in all 100 different sub classes of particles defined by both a) size and b) density is produced. To particles of each sub class a different ligand is bound to provide 100 unique combinations of ligand and particle.

20000 different ligands are to be tested. To this end, the 100 particle subclasses are used to provide a first lot of 100 sets of 100 particle-ligand combinations, which yields 10000 combinations. Furthermore, a second lot of 100 sets of 100 particle-ligand combinations, but with 10000 other ligands than in the first lot are made. In one and the same well one set of 100 particle-ligand combinations from the first lot is combined with twice as much (total number of particles or weight of the mixture or some other measure of quantity) from the second lot. This means that for each individual particle (having a unique size and density), there will be two possible ligands. The well is then incubated with a target molecule suitably marked.

After washing the wells to remove the non-bound target molecules, it is found that the size fraction 30-40 μm exhibits fluorescence (see Fig 2 which is a schematic representation of the contents of the well). It is also determined by simple counting or by measuring a total intensity of the fluorescence, that the number of fluorescing particles is twice as many as would have been the case if the target had reacted with particles from the first lot, and thus it must be from the second lot.

A separation by density through centrifugation is performed as in Example 1, yielding 10 bands, one of which is fluorescing (density 1,01). Again, all information needed to determine which ligand has reacted is available.

Example 3

In this example it is demonstrated that the number of possible ligands to test can be increased also by mixing the particle classes in an intelligent way.

Particles of three different particle sizes, average diameters being 15 μm, 25 μm and 55 μm respectively are used and 18 different ligands (designated A-S) are bound to these particles, thus forming 54 different combinations of ligand/particle. Thus, a combination of ligand A with a particle having the diameter 15 μm is designated A-15, ligand B bound to a 55 μm particle is designated B-55 etc.

Mixtures of these ligand-particle aggregates are prepared according to the following protocol:

Mixture No.	Composition (ligand/particle size)
1	A-15; B-25; C-55; D-15; E-25; F-55
2	G-25; H-55; I-15; K-25; L-55; M-15
3	N-55; O-15; P-25; Q-55; R-15; S-25
4	A-15; B-25; C-55; D-25; E-55; F-15
5	G-25; H-55; I-15; K-55; L-15; M-25
6	N-55; O-15; P-25; Q-15; R-25; S-55

According to this protocol, a combination can occur either in one well only, or in two different wells. For example the combination A-15 occurs in well No. 1 and 4, whereas combination D-15 occurs only in well No. 1.

These mixtures are placed in one well each (designated with the same number as the mixture numbers) of a micro titer plate, thus in six wells. The mixtures are incubated with one target substance, suitably marked with a fluorescent moiety. After completed incubation the wells are washed with water to remove all unbound target substances.

It is found that wells No. 2 and 5 exhibited fluorescence, and that the fluorescent particles have a diameter of 25 μm by image analysis. From this information it can be concluded that ligand G has bound to the target molecule, since the only combination with a particle of diameter 25 μm common to well 2 and 5 is G-25. This being an extremely simple example, it is appreciated that the protocol of mixing can be much more sophisticated and involved, in order to distinguish one reacting species among a large number of possibilities.

In Fig. 3 a typical image viewed in a fluorescence microscope is shown. As can be clearly seen it is possible even with ocular inspection to distinguish three different particle sizes (in this case for illustrative purpose, all particles fluoresce in order to be able to see them; in a real run of course only one particle size should be visible). An image analysis by computer yields the result shown in Fig. 4, wherein also the distribution of sizes within each nominal class can be seen.

In a further development of the method, the mixtures of ligand-particle combinations are incubated with two or more target molecules. To this end the targets are suitably marked with distinguishable markers, such as fluorescent moieties exhibiting fluorescence of different wave lengths.

What is claimed is:

1. A method of identifying one or more substances having affinity for a given target substance, comprising:

providing a set of particle classes, each said particle class being distinguishable from another class by at least one physical property, each particle class being comprised of at least two subclasses, wherein each subclass is distinguishable from another subclass by another physical property, different from the property of said particle classes, each subclass comprising particles having at least one of said substances attached to the surface thereof as a ligand, said ligands being different from ligands attached to particles of other particle classes or subclasses;

combining a plurality of sub-classes to form at least one mixture,

distributing the mixtures in separate vessels;

exposing each mixture to said target substance;

washing away all target substance not having bound to any ligand; and

identifying to which particle sub-class(es) said target substance actually has(have) bound, by the following steps:

identifying in which vessel or vessels target substance has bound to particles

in the mixture present in said vessel or vessels, identifying to which particle class said target substance has bound; and

identifying to which particle sub-class said target substance has bound.

2. The method as claimed in claim 1, wherein each particle class is characterized by one of the properties in the group size, density, color and shape.

3. The method as claimed in claim 2, wherein each particle sub-class is characterized by one of the properties in the group size, density, color and shape, but is different from the property characterizing the particle class.

4. The method as claimed in claim 1, wherein the mixtures are formed by mixing two particle classes in a ratio such that the difference in number of one class with respect to the other is detectable in the identifying step.

5. The method as claimed in any preceding claim, wherein the target substance is marked so as to be detectable.

6. The method as claimed in claim 5, wherein the marking is performed by attaching a moiety selected from the group consisting of a fluorescent moiety, a radioactive moiety, a colored moiety.

7. The method as claimed in claim 5, wherein the target substance reacts with the ligand to which it binds to provide a detectable effect, such as fluorescence or color.

8. The method as claimed in any preceding claim, wherein the identification is performed by ocular inspection under microscope.

9. The method as claimed any preceding claim, wherein the identification is performed by image analysis with a computer.

10. The method as claimed in any preceding claim, wherein said mixture is exposed to at least two target substances.

11. A library of different ligands, comprising particles belonging to a plurality of classes, each particle class being distinguishable from another class by at least one physically distinguishable property, each particle class being comprised of at least two subclasses, wherein each subclass is distinguishable from another subclass by another physical property,

different from the property(ies) of said particle classes, those particles belonging to one and the same sub-class having at least one type of ligand attached to their surface.

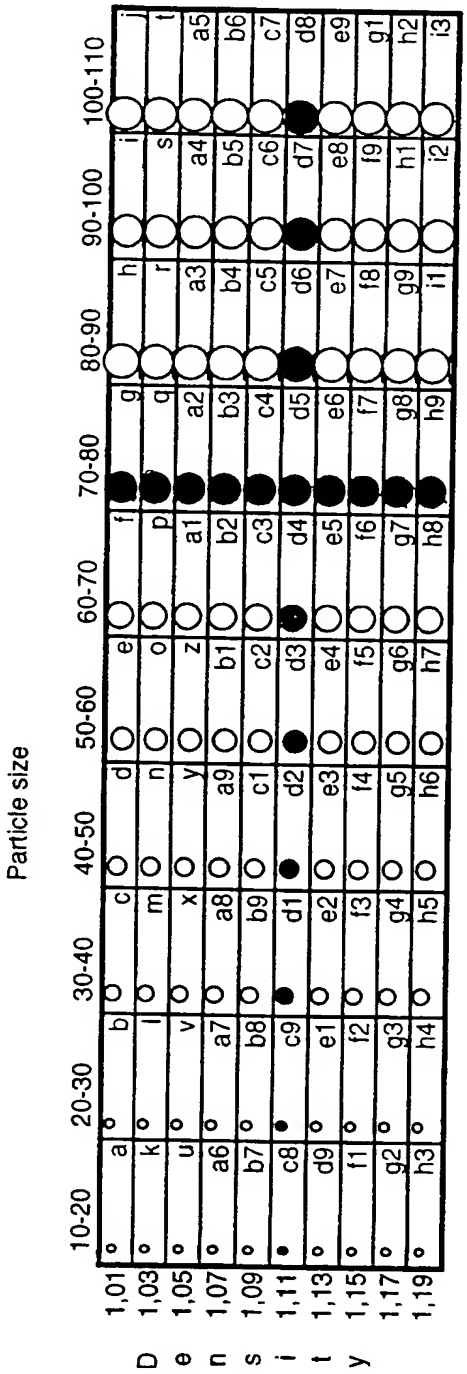
12. The ligand library as claimed in claim 11, wherein one of said properties of the particle classes or sub-classes is the size, suitably the diameter of the particles.

13. The ligand library as claimed in claim 11, wherein one of said properties of the particle classes or sub-classes is the density of the particle.

14. The ligand library as claimed in claim 11, wherein one of said properties of the particle classes or sub-classes is the shape of the particle.

15. The ligand library as claimed in claim 11, wherein one of said properties of the particle classes or sub-classes is the color of the particle.

16. The use of a ligand library as claimed in claim 11 for screening purposes.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30-40
40-50
50-60
60-70
70-80
80-90
90-100
100-110
Density
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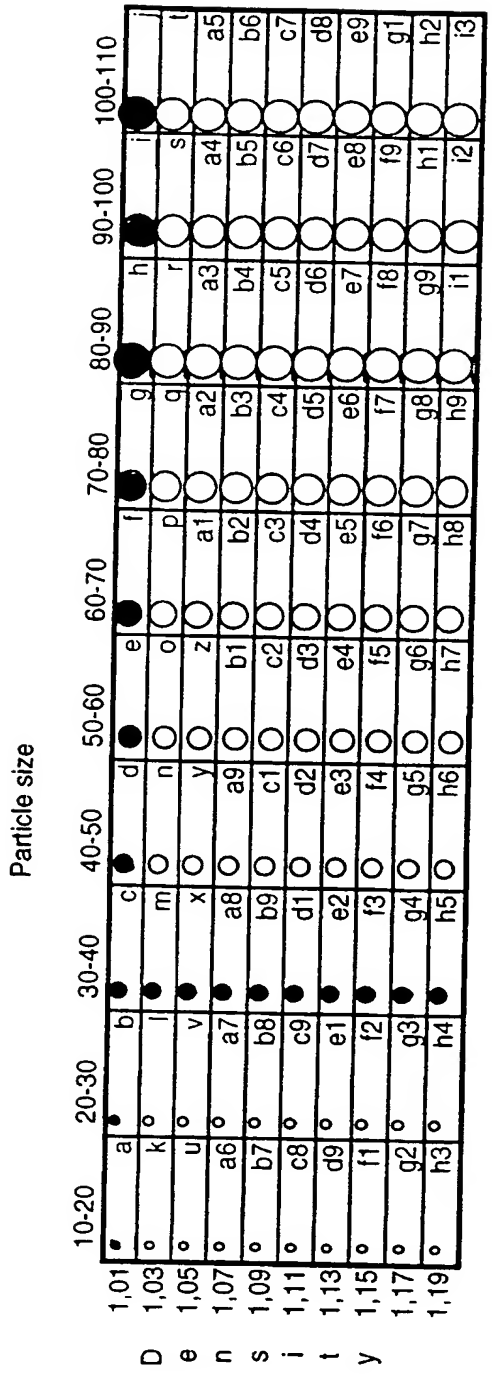
1,11
1,13
1,15
1,17
1,19

Fig. 1


Particle size
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50-60
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90-100
100-110
Density
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1,19

Fig. 2

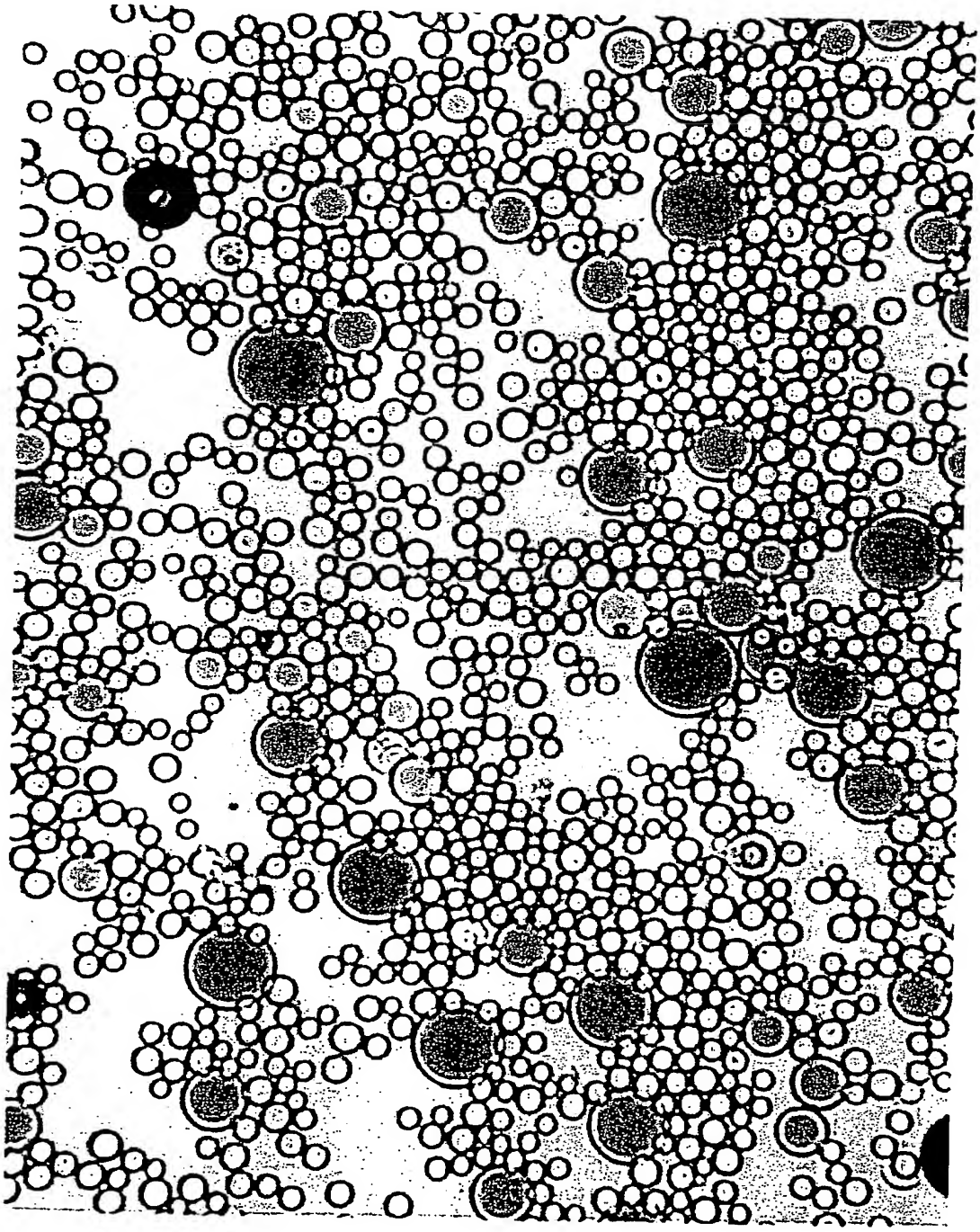

Fig. 3

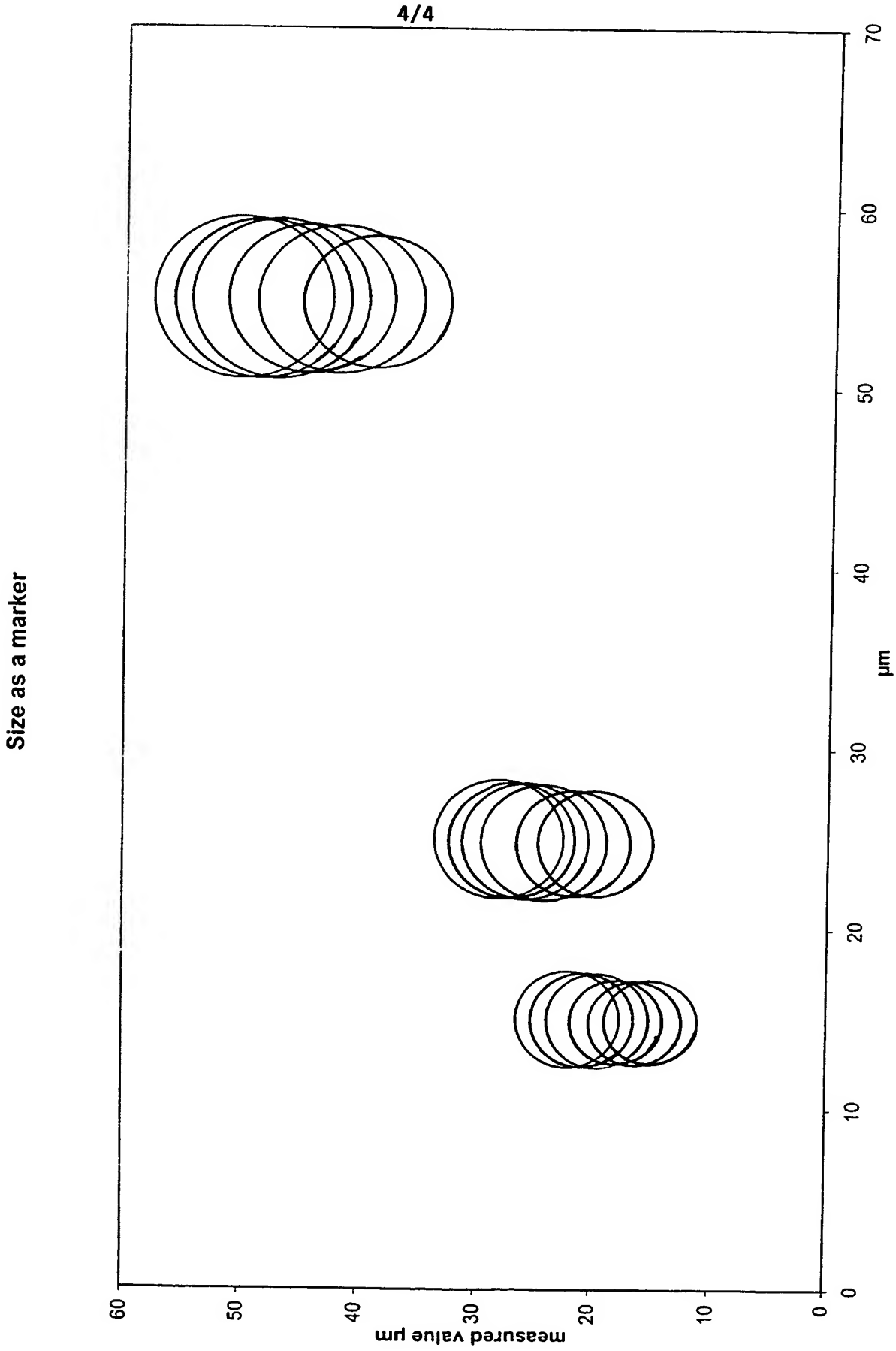

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01174

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/543, C12Q 1/68
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N, C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	Biopolymers (Peptide Science), Volume 37, 1995, (Tucson), Michal Lebl et al, "One-Bead-One-Structure Combinatorial Libraries" page 177 - page 198	1-16
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 2000	06 -11- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl-Olof Gustafsson Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01174

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	WO 9622531 A1 (HÄNNINEN, PEKKA), 25 July 1996 (25.07.96), page 7 - page 8	1-16
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Y	EP 0440193 A2 (FUJIREBIO INC.), 7 August 1991 (07.08.91)	1-16
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International application No.
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